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Supplementary Catechins Attenuate Cooking-Oil-Fumes-Induced Oxidative Stress in Rat Lung

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Abstract

Cooking-oil-fumes containing toxic components may induce reactive oxygen species (ROS) to oxidize macromolecules and lead to acute lung injury. Our previous study showed that a decaffineated green tea extract containing (+)-catechin, (–)-epicatechin, (+)-gallocatechin, (–)-epigallocatechin, (–)-epicatechin gallate, and (–)-epigallocatechin gallate can inhibit oxidation, inflammation, and apoptosis. We determined whether the catechins supplement may reduce cooking-oil-fumes-induced acute lung injury in rat. In the urethane-anesthetized Wistar rat subjected to 30-120 min of cooking-oil-fumes exposure, blood ROS significantly increased in the recovery stage. After 30-min cooking-oil-fumes exposure, the enhanced blood ROS level further increased in a time-dependent manner during the recovery stage (321 ± 69 counts/10 s after 1 h, 540 ± 89 counts/10 s after 2 h, and 873 ± 112 counts/10 s after 4 h). Four hours after 30-min cooking-oil-fumes exposure, lung lavage neutrophils and ROS as well as lung tissue dityrosine and 4-hydroxy-2-nonenal increased significantly. Two weeks of catechins supplememnt significantly reduced the enhanced lavage ROS, lung dityrosine and 4-hydroxy-2-nonenal level. Cooking-oil-fumes-induced oxidative stress decreased lung Bcl-2/Bax ratio and HSP70 expression, but catechins treatment preserved the downregulation of Bcl-2/Bax ratio and HSP70 expression. We conclude that catechins supplement attenuates cooking-oil-fumes-induced acute lung injury *via* the preservation of oil-smoke induced downregulation of antioxidant, antiapoptosis, and chaperone protein expression.

Key Words: catechins, reactive oxygen species, HSP70, cooking-oil-fumes, apoptosis, lung

Introduction

Epidemiological studies have indicated an increased risk of respiratory tract injury among cooks and bakers (8, 24, 30). Previous study by Wu and Yen (30) has demonstrated that mutagenic aldehydes were enriched in the cooking-oil-fumes collected from peanut oil heated to its smoke point and produced genotoxicity and oxidative stress to lung carcinoma cells. Ho *et al*. (17) implicated these aldehydes were

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an oxidation product formed by linoleic acid and other polyunsaturated fatty acids. For example, trans-, trans-2,4-decadienal, a specific type of dienaldehyde, is abundant in heated oils and has been associated with lung adenocarcinoma development in women due to their exposure to oil fumes during cooking (1). Cultured human bronchial epithelial cells with this dienaledhyde enhanced an increase in reactive oxygen species (ROS) production and pro-inflammatory cytokines TNFα and IL-1β, and a decrease in GSH/GSSG ratio (glutathione status) expression (1, 9). However, as we know, the *in vivo* model for exploring cooking-oil-fumes-induced oxidative stress in lung injury of the rat has not yet been defined and well established.

Excess productions of ROS contributing to abnormal signal transduction or cellular dysfunction trigger the cascade of inflammation, apoptosis, autophagy, and necrosis (4-6, 28). Recent findings have demonstrated that antioxidant supplementation like N-acetylcysteine inhibits the progression of lung epithelial cell injury and inflammation (1). Green tea extracts containing (+)-catechin (C), (–)-epicatechin (EC), (+)-gallocatechin (GC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), and (–)-epigallocatechin gallate (EGCG) are considered to be strong antioxidants (25) exerting protective effects against cancer and inflammatory and cardiovascular diseases (16). Catechins can inhibit pro-inflammatory and pro-apoptotic oxidative injury *via* the action of reduction in ROS production, nuclear factor-κB (NF-κB) and activated protein-1 (AP-1) translocation, and intercellular adhesion molecule 1 (ICAM-1) expression as well as proapoptotic mechanisms (2, 32). Furthermore, in scavenging plasma H_2O_2 and HOCl activity, catechins are more effective than vitamin C and E (18) and can efficiently prevent carcinogenesis (20). We therefore speculate that catechins supplement may provide an alternative approach to protecting against cooking-oil-fumes-induced acute lung injury in rats.

Materials and Methods

Animal Surgery

Female Wistar rats (190-240 g, mean body weight 245 ± 15 g) were housed at the Experimental Animal Center, National Taiwan University, at a constant temperature and with a consistent light cycle (light from 07:00 to 18:00 o'clock). Food and water were provided *ad libitum*. All surgical and experimental procedures were approved by National Taiwan University College of Medicine and College of Public Health Institutional Animal Care and Use Committee and are in accordance with the guidelines of the National Science Council of the Republic of China (NSC 1997).

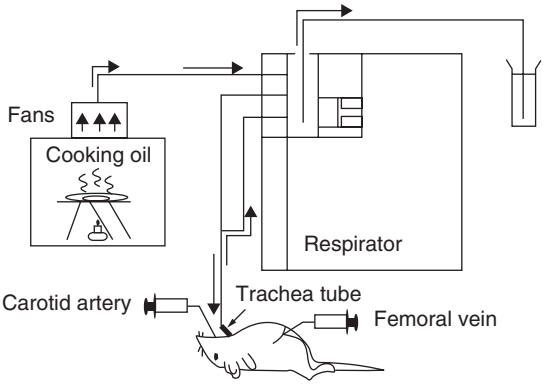

Fig. 1. A set up for acute exposure of cooking-oil-fumes to the urethane-anesthetized Wistar rat. The cooking-oil-fumes are directly delievered to the lung of an anesthetized rat by a respirator.

On the experiment day, the rat was anesthetized with subcutaneous urethane (1.2 g/kg) throughout the entire experiment. The body temperature was kept at 36.5-37.0°C by an infrared light and was monitored with a rectal thermometer. Each rat was cannulated with a PE-200 tubing. A PE-50 catheter was placed in the left carotid artery to assess blood gas and pH data by a gas analyzer (Nova blood gas analyzer, SP5, Hamburg, Germany), heart rate and arterial blood pressure by an ADI system (PowerLab/16S, ADI Instruments, Pty Ltd, Castle Hill, Australia) with a transducer (P23 1D, Gould-Statham, Quincy, MA, USA) and for blood sampling. Another PE-50 tube was inserted into the femoral vein for anesthetics supplement.

A Set Up for Transfer of Heated Cooking-Oil-Fumes to the Rat

Cooking-oil-fumes were collected as described previously (30). The commercial cooking oil (soybean oil) was purchased from traditional markets in Taipei, Taiwan, ROC. The soybean oil (5 g) was poured into an iron pot (12 cm in diameter) on a heater, and the heating temperature was controlled at the smoke point ±10°C. The cooking-oil-fumes from the heated oil (220 ± 10°C) were pumped into the animals *via* the trachea cannuation with an animal respirator (Fig. 1) and a piece of filter paper (capillary 3 μm, Unique Pretty Industries, Gary, IN, USA) was placed 50 cm above the oil surface at a flow rate of 2 l/min for measurement of lipid peroxides in the cooking-oil-fumes. The concentration of lipid peroxides as indicated by malondialdehyde (MDA) was determined

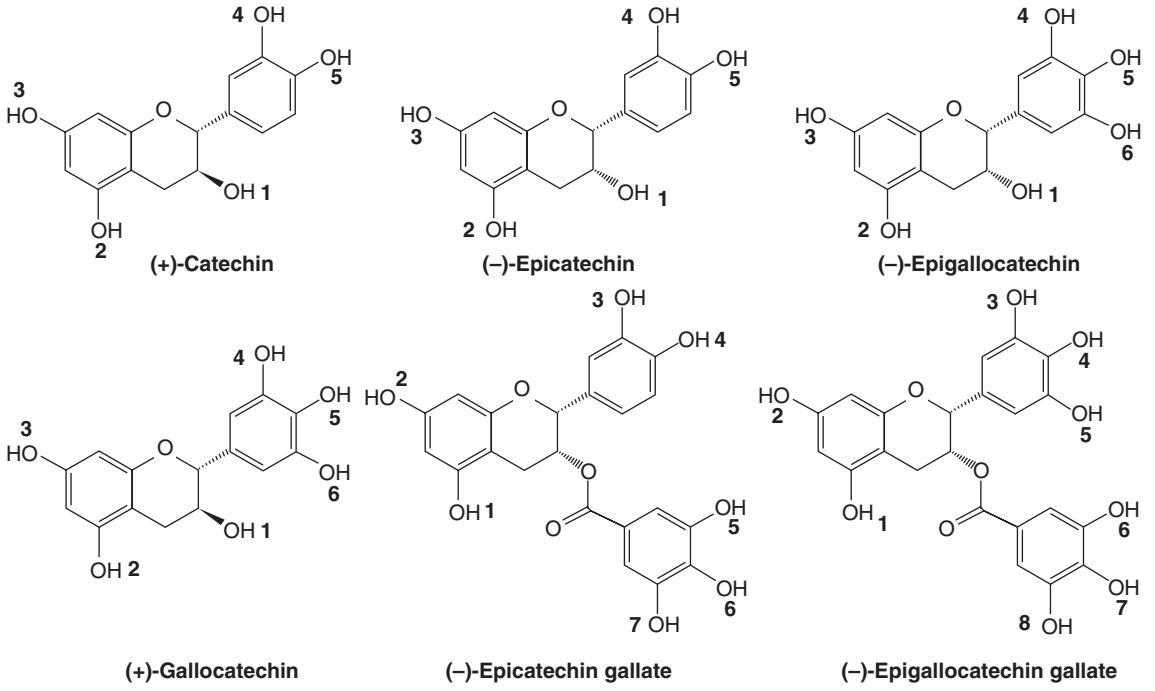

Fig. 2. Structures of six catechins. The number indicates the –OH amount in each catechin.

using the Yagi method (31). The MDA level was increased in the filter paper with cooking-oil-fumes treatment (4.1 ± 0.3 pmol/g of paper weight) when compared to that in the non-cooking oil treatment (0 ± 0 pmol/g of paper weight). The animals were subjected to cooking-oil-fumes for 30, 60, 90, and 120 min, which was followed by a recovery period at the end of the exposure and then stopped cooking-oil-fumes exposure as the recovery period. The anesthetized animals were mechanically ventilated with tidal volume (7-10 ml/kg body weight) and frequency (45-60 pumps/min). The setting condition of tidal volume and frequency was adjusted by the parameters of blood gas and pH. The arterial pressure and heart rate were also monitored before or after serial blood sampling during the experiment. In some rats, catechins supplement were administered at a dose of 4 mg/kg/day intragastrically for two weeks or two months. Decaffeinated catechins was purchased from Numen Biotech Co., Ltd. (Taipei, Taiwan, ROC); the catechins consisted of 328 mg/g of epigallocatechin gallate, 152 mg/g of epicatechin gallate, 148 mg/g of gallocatechin gallate, 132 mg/g of epicatechin, 108 mg/g of epigallocatechin, 104 mg/g of gallocatechin, and 44 mg/g of catechin (Fig. 2).

Blood ROS Measurement

A series of blood samples (0.2 ml) from the carotid artery were obtained immediately after cooking-oil-fumes exposure for various times durations. The blood samples were immediately determined for lucigenin (a superoxide anion probe)-amplified chemiluminescence (ROS level) as described previously (4, 5). Briefly, ROS was measured in a completely dark chamber of the Chemiluminescence Analyzing System (CLD-110, Tohoku Electronic In. Co., Sendai, Japan). The real-time displayed chemiluminescence signal was recognized as ROS level from the blood samples. The total amount of ROS was calculated by integrating the area under the curve and subtracting the background level. The assay was performed in duplicate for each sample and was expressed as ROS counts/10 sec. The mean and standard error (SE) of the ROS level of each sample was calculated.

Bronchoalveolar Lavage

The protocols for bronchoalveolar lavage of animals were followed as described previously (12). Four h after 30-min exposure to cooking-oil-fumes, the chest cavity was opened by a midline incision, the left main-stem bronchus was ligated, and the right lung was lavaged with one aliquot of Ca^{2+}/Mg^{2+}-free phosphate buffer saline (pH 7.4). The volume of the aliquot was equal to 35 ml/kg of body weight (~90% of total lung capacity). The aliquot was instilled into the lungs three times before final collection. The bronchoalveolar lavage was immediately determined for ROS as described above. The neutrophil number in the bronchoalveolar larvage was determined

with a cell counting analyzer (Sysmex TM K-1000, TOA Medical Electronics Co., LTD., Kobe, Japan).

Measurement of Oxidized Amino Acids and Lipid Peroxidation in the Lung

In the presence of H_2O_2, tyrosyl radicals generated by myeloperoxidase may participate in the oxidation of lipids and proteins (14) and cross-link to give fluorescent adducts, dityrosine, which can be determined with a fluorometer (Hitachi F-2500, Tokyo, Japan) (29). We measured the dityrosine level in the 50 mg of lung tissues homogenate with phosphate buffer saline from the control and 4 h after 30-min cooking-oil-fumes exposure with or without catechins, a green tea extract, supplement. The homogenate was centrifuged at 5,000 × g for 10 min at 4°C, and the supernatant was collected to determine the dityrosine level.

Measurement of 4-Hydroxy-2-Nonenal (4-HNE) Levels in the Lung

Lung tissues were obtained at 4 h after 30-min cooking-oil-fumes exposure. The 4-HNE levels (22) were determined in the 50 mg of lung tissue homogenate by using the lipid peroxidation assay kit (Calbiochem, San Diego, CA, USA). The lung tissue homogenates were suspended in 200 μl of 20 mM Tris-HCl, pH 7.4, containing 5 mM butylated hydroxytoluene, and kept frozen at –70°C until assayed. To each sample, 650 μl of N-methyl-2-phenylindole and 150 μl of 15.4 M methanesulfonic acid were added. The reaction mixture was vortexed and incubated at 45°C for 60 min. After centrifugation at 15,000 × g for 10 min, the absorbance of the supernatant was determined at 586 nm. The levels of 4-HNE were determined from standard calibration curve constructed using 4-HNE diethylacetal in methanesulfonic acid. The values were expressed as μmol 4-HNE/mg protein.

Immunoblotting of Bax, Bcl-2 and HSP70 in the Lung

The expression of Bax, Bcl-2, and HSP70 of lung tissues obtained at 4 h after 30-min cooking-oil-fumes exposure was evaluated by western immunoblotting and densitometry as described previously (4, 32). Briefly, the total proteins were homogenized with a prechilled mortar and pestle in the extraction buffer, which consisted of 10 mM Tris-HCl (pH 7.6), 140 mM NaCl, 1 mM phenylmethyl sulfonyl fluoride, 1% Nonidet P-40, 0.5% deoxycholate, 2% β-mercaptoethanol, 10 μg/ml pepstatin A, and 10 μg/ml aprotinin. The mixtures were homogenized completely by vortexing and kept at 4°C for 30 min. The homogenate was centrifuged at 12,000 × g for 12 min at 4°C, the supernatant was then collected, and the protein concentrations were determined by the Bio-Rad Protein Assay (Bio-Rad laboratories, Hercules, CA, USA).

Ten μg of protein was electrophoresed as described below. The polyclonal rabbit antihuman Bax (Chemicon International, Temecula, Temecula, CA, USA), monoclonal mouse antihuman Bcl-2 (Biovision, Mountain View, CA, USA), mouse anti-HSP70 (Transduction Laboratories, Lexington, KY, USA), and monoclonal mouse antimouse β-actin (Sigma, Saint Louis, MO, USA) were used at 1:1000. All of these antibodies cross-react with the respective rat antigens. Proteins on SDS-PAGE gels were transferred to nitrocellulose filters and stained as described (4).

Statistical Analysis

All values were expressed as means ± SEM. Differences between groups were evaluated by paired Student's *t*-test. One-way analysis of variance was used to establish the differences among the groups. Intergroup comparisons were made by Duncan's multiple-range test. Differences were regarded as significant at $P < 0.05$.

Results

Baseline Physiological Parameters

The levels of partial pressure of O_2 and CO_2, and pH value from carotid arterial blood in baseline control stage of anesthetized rats were 97 ± 3 mmHg, 30 ± 2 mmHg, and 7.42 ± 0.02. During cooking-oil-fume exposure of our model, the levels in partial pressure of O_2 and CO_2, and pH value from the arterial blood were 92 ± 4 mmHg, 33 ± 2 mmHg, and 7.38 ± 0.03. After cooking-oil-fumes exposure, the levels of partial pressure of O_2 and CO_2, and pH value from carotid arterial blood were 98 ± 3 mmHg, 31 ± 2 mmHg, and 7.41 ± 0.02. In our study, the level of mean arterial blood pressure was similar before and during cooking-oil-fumes exposure (110 ± 4 mmHg *vs*. 108 ± 5 mmHg). The heart rate was also similar before and during cooking-oil-fumes exposure (386 ± 15 beats/min *vs*. 369 ± 20 beats/min). These data suggest that acute cooking-oil-fumes exposure did not affect the rat hemodynamic responses.

Different Time of Exposure of Cooking-Oil-Fumes Enhanced ROS Production

We found that the baseline value of blood ROS in the control rats was around 40-100 counts/10 sec. In response to cooking-oil-fumes exposure, there was

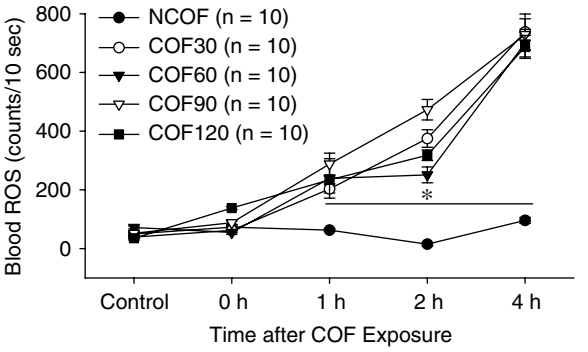

Fig. 3. Effect of cooking-oil-fumes (COF) exposure time on blood ROS sampled at baseline stage, 0, 1, 2, and 4 h after COF exposure in urethane-anesthetized rats. Non-cooking-oil-fumes (NCOF) treatment did not affect blood ROS level. COF exposure at 30 min (COF30), 60 min (COF60), 90 min (COF90), and 120 min (COF120) significantly enhanced blood ROS level at 1-4 h after COF exposure in a time-dependent pattern. 0 h is the time just following exposure for 30-120 min of cooking-oil-fumes. *, $P < 0.05$ when compared to the respective baseline control value.

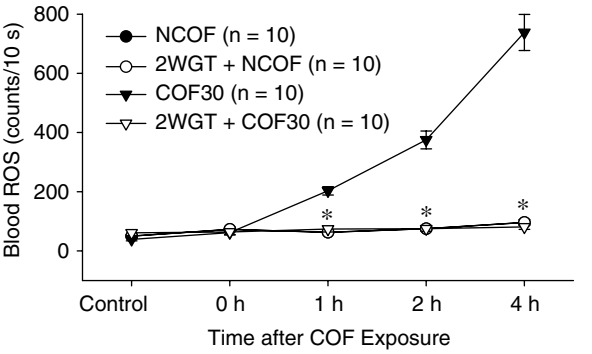

Fig. 4. Effect of 2 weeks of catechins, a green tea extract (2wGT), on 30 min of cooking-oil-fumes (COF30)-exposure-enhanced blood ROS sampled at baseline stage, 0, 1, 2, and 4 h after COF exposure in urethane-anesthetized rats. Non-cooking-oil-fumes (NCOF) treatment did not affect blood ROS level. 2WGT significantly decreased COF30-enhanced blood ROS level. *, $P < 0.05$ when compared to the respective COF30 value.

no increase in blood ROS in all groups of rats. After cooking-oil-fumes exposure, the blood ROS increased time-dependently to 800 counts/10 sec at 4 h in the recovery period. As shown in Fig. 3, the blood ROS value was similar in the control rats without cooking-oil-fumes exposure at all measured time points. Our results also showed that 30-120 min of exposure of cooking-oil-fumes displayed a similar but not increased blood ROS level (Fig. 3). However, after the stop of cooking-oil-fumes stimulation, the blood ROS value significantly ($P < 0.05$) increased in the recovery period in a time-dependent manner.

Catechins Supplement Decreased Cooking-Oil-Fumes-Enhanced Blood ROS

As shown in Fig. 4, 2 weeks of catechins pretreatment significantly decreased 30 min of cooking-oil-fumes-exposure-enhanced blood ROS amount in urethane-anesthetized rats. Catechins pretreatment did not affect the baseline level of blood ROS in rats without cooking oil exposure.

Catechins Decreased Cooking-Oil-Fumes-Enhanced Bronchoalveolar Lavage ROS, Lung Dityrosine and 4-HNE Levels

For more specific confirmation of cooking-oil-fumes-induced oxidative stress in the lung, we measured neutrophils and ROS amount in the bronchoalveolar lavage and the oxidative markers, dityrosine and 4-HNE, in the lung tissue. The number of neutrophils in the lavage increased significantly 4 h after cooking-oil-fumes exposure. We also found that 4 h after cooking-oil-fumes exposure significantly increased bronchoalveolar lavage ROS amount and increased dityrosine and 4-HNE in the lung (Fig. 5). Catechins supplement significantly decreased the number of lavage neutrophils compared with the exposure group without catechins (Fig. 5). Two weeks of catechins supplement significantly reduced 30 min of cooking-oil-fumes-exposure-enhanced lavage ROS and lung dityrosine and 4-HNE levels in urethane-anesthetized rats.

Catechins Preserved Cooking-Oil-Fumes-Decreased Bcl-2 and HSP70 Expression

As shown in Fig. 6, we found that 4 h after 30-120 min of cooking-oil-fumes exposure significanty enhanced Bax protein expression and decreased Bcl-2 and HSP70 protein expression in rat lung. The enhanced Bax and decreased Bcl-2 expression significantly amplified the Bax/Bcl-2 ratio and promoted the proapoptotic pathway in rat lung subjected to cooking-oil-fumes. Two weeks and two months of catechins supplement can inhibit lung Bax enhacement and preserve Bcl-2 and HSP70 protein expression in rats with cooking-oil-fumes exposure.

Discussion

In the present study, we developed an *in vivo* model for exploring the detrimental effect of cooking-oil-fumes-evoked oxidative stress in the rat. Our results showed that 30 min of cooking-oil-fumes inhalation enhanced blood ROS and bronchoalveolar

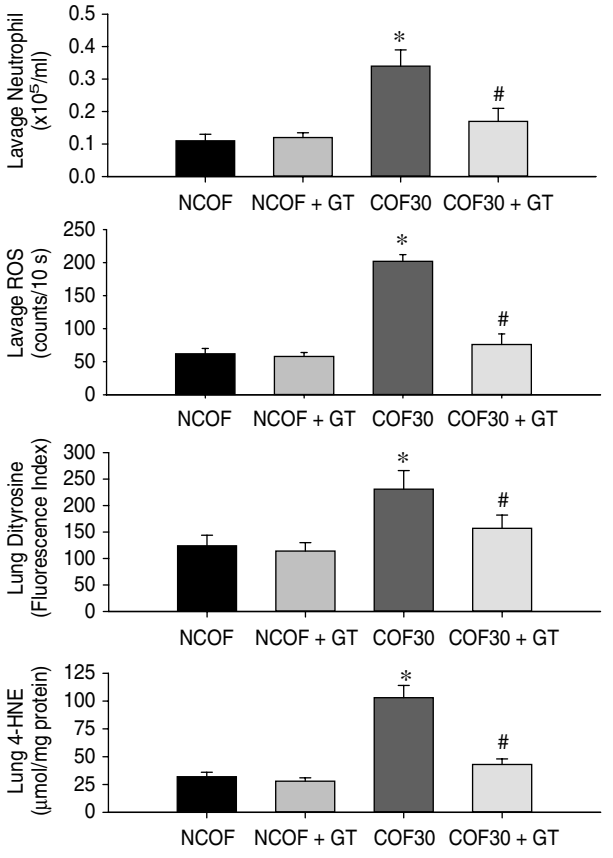

Fig. 5. Effect of 2 weeks of catechins, a green tea extract (GT), supplement on 30 min of cooking-oil-fumes (COF30)-exposure-enhanced lavage ROS and lung dityrosine and 4-HNE levels in urethane-anesthetized rats. Four h after COF30 exposure significantly increased lavage ROS and protein oxidation (dityrosine) and lipid peroxidation (4-HNE) products in the lung when compared to the non-cooking-oil-fumes (NCOF) group. *, $P < 0.05$ when compared to the NCOF value. #, $P < 0.05$ COF30 + GT *vs*. COF30 group.

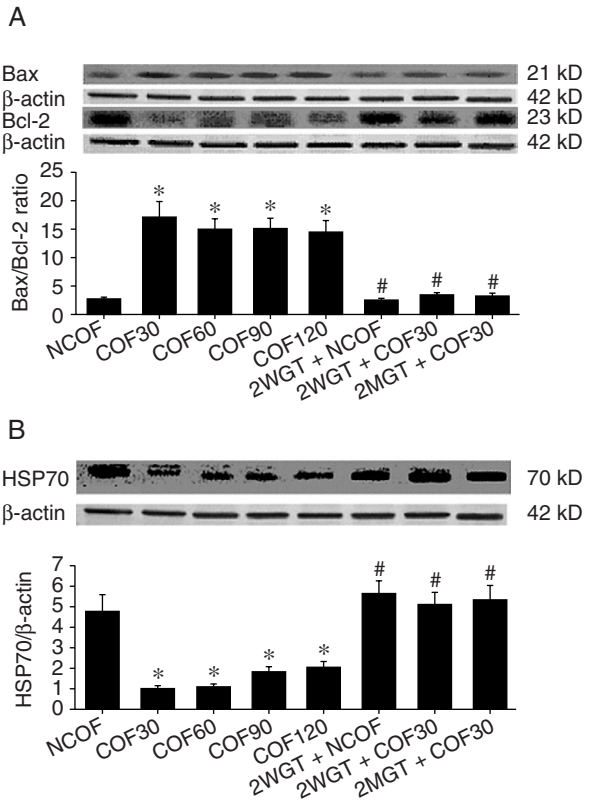

Fig. 6. Effect of catechins, a green tea extract (GT), supplement on lung Bax, Bcl-2, HSP70 protein expression in rats with cooking-oil-fumes (COF) stimulation. Western blot shows that 30 min (COF30), 60 min (COF60), 90 min (COF90) and 120 min (COF120) of COF stimulation increased Bax, but depresses Bcl-2 and HSP70 protein expression in rat lung 4 h after COF stimulation. The increased Bax/Bcl-2 ratio and decreased HSP70 expression by COF30 were reversed by 2 week-(2WGT+COF30) and 2 month-GT (2MGT + COF30) treatment. *, $P < 0.05$ when compared to the no cooking oil fumes (NCOF) treated value. #, $P < 0.05$ *vs*. COF30 group.

lavage ROS level. The increased ROS oxidized lung tissue by an elevated level of dityrosine and 4-HNE products, and increased Bax/Bcl-2 ratio for proapoptotic pathways and decreased HSP70 chaperone protection pathways. Catechins, a green tea extract, exerted ROS scavenging activity to decrease oxidative stress and Bax/Bcl-2 ratio and preserve HSP70 chaperone protein expression in the lung.

Theory and empirical studies have demonstrated that high temperature and the repeated use of oil lead to the breakdown of some fatty acids and the formation of many toxic polymer compounds and peroxides (10). The formation of free oxygen radicals is a normal process in all living cells, during the reduction of oxygen to water, leading, however, to the production of a number of peroxide compounds, such as hydrogen peroxide and lipid peroxides, which are toxic and cause great disruption to living cell metabolism (13). ROS have been shown to play an important role in numerous forms of inflammation (16). The gas and cooking oil smoke contain oxidants and free radicals that may cause the sequestration of neutrophils from the pulmonary microcirculation as well as an accumulation of macrophages in respiratory bronchioles (1, 9). In addition, alveolar macrophages and neutrophils have the potential to produce large amounts of reactive oxygen intermediates. In our data, we found that 30-120 min of exposure of cooking-oil-fumes only enhanced blood ROS in the recovery period but not before or at the termination of 30-120 min of oil fume exposure. Thirty min of cooking-oil-fume exposure might have accumulated a saturated level of toxical substances in the lung of our model and therefore displayed a similar blood ROS when

compared to 60-120 min of oil fume exposure groups. A delayed increase in blood ROS might have been caused by the increased amount or activation of blood leukocytes, which were stimulated by the accumulation of toxic polymer compounds and peroxides in the lung in the recovery stage. We suggest that a low oxygen tension during oil fumes stimulation possibly occurred in our closed setup model and a return of oxygen tension after the stop of oil fumes stimulation led to an enhancement of blood ROS. However, this implication requires to be further determined in the future. Our unpublished data found that during oil fumes stimulation, the bronchoalveolar lavage ROS and neutrophiols were not immediately enhanced. Our data showed that 4 h after 30 min of cooking-oil-fumes exposure significantly increased neutrophils and ROS amount in the bronchoalveolar lavage and increased protein oxidation product, dityrosine and lipid peroxidation product, 4-HNE in the lung tissue. Increased neutrophil amount or activated neutrophils can release ROS like H_2O_2 and HOCl which oxidize lipid and protein and impair tissue (18). Oxidants, either inhaled or generated by inflammatory cells, have been implicated in the inflammatory process in the lungs (1, 12).

Long-term tobacoo smoking is the primary cause for lung injury (3); however, epidemiological studies have also shown that chronic exposure to cooking-oil-fumes is another risk factor for lung damage (19, 21, 23, 33). This population-based case-control study confirmed that exposure to indoor air pollution from Chinese-style cooking, especially cooking unrefined rapeseed oil at high temperatures in woks, may increase the risk of lung injury (33). The major toxic compound (trans-trans-2,4-decadienal) of cooking peanut oil fumes can apparently produce $O_2^{-\cdot}$, H_2O_2, OH. in a phosphate buffer, and the generated intracellular ROS lead to cytotoxicity and oxidative DNA damage (indicated by 8-hydroxy-2'-deoxyguanosine formation), and decrease glutathione content, and the activities of antioxidative enzymes such as GSH reductase, GSH peroxidase and GSH S-transferase (30). Hung *et al*. (19) showed that cooking-oil-fumes and their two major components, benzo[a]pyrene and 2,4-decadienal, significantly affect apoptosis proteins and caspase-3 expression. Our data also showed that cooking-oil-fumes containg high concentration of MDA may damage lung tissue in the rat. The treatment with vitamin C and N-acetylcysteinine partially prevented trans-2,4-decadienal-induced cell cytoxicity and proliferation response (11, 27). In the present study, we further found "pretreatment with catechins (a green tea extract) prevents cooking-oil-fumes-induced oxidative stress and apoptosis in lungs.

We used a well-established enhanced CL method (4-6) to study ROS, especially $O_2^{-\cdot}$ production in the lung lavage and blood. This method has been well established for measurement of ROS production in cultured cells, the whole-blood system, isolated perfused organs, urinary bladder, and kidney *in vivo* (4-6). We showed that the level of lucigenin-enhanced chemiluminescence detected from the blood and bronchoalveolar lavage ROS increased 1-4 h after cooking-oil-fumes exposure. A previous *in vitro* study indicated that the cytotoxicity, DNA cross-links, DNA single strand breaks, and malondialdehyde were increased in rat type II lung cells exposed to cooking-oil-fumes, whereas the GSH status decreased significantly and time-dependently with exposure doses of cooking-oil-fumes (27). We suggest that the cooking-oil-fume and its toxic components have a time-dependent pathway to trigger the enhanced oxidative stress in the lung. We also found that 30 min of cooking-oil-fumes exposure displayed an increased level similar to that of 60, 90, or 120 min of cooking-oil-fumes exposure. This result indicated that 30 min of exposure may have a maximal effect in the induction of oxidative stress in rat. The increased ROS evoked by cooking-oil-fumes oxidized proteins and lipids in the lung as measured by dityrosine and 4-HNE. By employing a lavage ROS measurement, we suggest that the cellular source of ROS, especially $O_2^{-\cdot}$ synthesis, was possibly from leukocytes and lung epithelial cells; however, the detailed information needs to be determined in the future.

The excess ROS generated from activated neutrophils *via* the myeloperoxidase system can produce lipid peroxidation products, MDA, phosphatidylcholine hydroperoxide, protein oxidation, dityrosine, and methylguanidine, as indirect indicators of ROS and/or free-radical activity (18). In the present study, increases in bronchoalveolar lavage neutrophils and ROS are associated with increases in lung dityrosine and 4-HNE. The increased ROS can trigger the translocation of NF-(B and AP-1 to nucleus to promote inflammatory signaling pathways leading to further production of ROS and apoptosis in the cells or tissues (4-6, 11). A previous study (9) has shown that heating products from three commercial cooking oils (soybean oil, sunflower oil, and lard) induced genotoxicity and cytotoxicity in the human lung carcinoma of pulmonary type II-like epithelium cell. The major alkenal mutagenic compounds (trans-trans-2,4-decadienal, trans-trans-2,4-nonadienal, trans-2-decenal, and trans-2-undecenal) contained in oil fumes can induce ROS, cytotoxicity and oxidative DNA damage and lead to a decrease in GSH contents and the activities of antioxidant enzymes such as GSH reductase, and GSH S-transferase (9).

Various kinds of antioxidants capable of decreasing NF-κB activity, ameliorating mitochondrial dysfunction, cytochrome c release and caspase-

3-mediated apoptosis, and decreasing inflammatory cell infiltration (4-6, 15, 32) have been shown to reduce tissue injury. Recently, ROS-enhanced pro-inflammatory NF-κB, AP-1, and ICAM-1 expression as well as promoted proapoptotic mechanisms, including increases in the Bax/Bcl-2 ratio, in CPP32 expression, in poly-(ADP-ribose)-polymerase cleavages, in DNA fragmentation, and in apoptotic cells in the liver can be inhibited by the catechins supplement (2, 32). The cotreatment of epigallo-catechin gallate resulted in complete protection of the hepatocyte apoptosis by suppressing the increases of caspase-3 in cytoplasm (32). Our results indicated that catechins, can efficiently reduce cooking-oil-fumes-evoked and ROS-mediated oxidative injury. The present data and previous reports (2, 15, 32) have indicated that green tea extracts containing catechins can protect tissues and cells against oxidative injury. We suggest that directly scavenging ROS activity, preserving several antioxidant enzymes and down-regulate Bax/Bcl-2 ratio in the lung tissue contribute to the protection. Our previous study has shown that administration of catechins inhibited NF-κB and AP-1 activation, reduced the expression of ICAM-1 at protein levels, and attenuated cellular apoptosis in the injuried tissues (32). Catechins can inhibit redox-sensitive transcription factors in cancer cells (7, 26) and damage of cells or tissues mediated by ROS (2, 5). Effective scavenging of ROS or maintenance of the cellular redox state by catechins may represent a useful therapeutic approach to limiting inflammation- and apoptosis-mediated injury by oil fumes.

In summary, the present study indicates that cooking-oil-fumes-induced ROS in the blood and lung reduce the antioxidant defense mechanism and trigger propaoptotic pathway in the lung, and pretreatment with catechins could prevent lung injury mediated by cooking-oil-fumes-evoked ROS.

Acknowledgments

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